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B. T. GALLOWAY, *Chief of Bureau.*

# A BIOCHEMICAL STUDY OF THE CURLY-TOP OF SUGAR BEETS.

BY

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# LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Plant Industry,
Office of the Chief,
*Washington, D. C., November 6, 1912.*

Sir: I have the honor to transmit herewith and to recommend for publication as Bulletin No. 277 of the series of this Bureau the accompanying manuscript entitled "A Biochemical Study of the Curly-Top of Sugar Beets," by Dr. H. H. Bunzel, Chemical Biologist, submitted by Dr. R. H. True, Physiologist in Charge of the Office of Drug-Plant, Poisonous-Plant, Physiological, and Fermentation Investigations.

The study of the oxidizing processes seen in the physiological and pathological behavior of living things has until recently suffered on account of the lack of a satisfactory method for accurately investigating these phenomena. With the recent development of improved means of studying these reactions, however, it has become possible to view these processes of plant and animal activity in a somewhat clearer light. The accompanying paper summarizes the results following the application of the manometric method to a study of an important and somewhat obscure condition of sugar beets. New light has been thrown not only on a number of features of the trouble itself but also on the oxidizing activities of the normal sugar beet. While these results do not establish the fundamental causes of the curly-leaf disease, they seem likely to introduce more fundamental considerations.

Respectfully,

B. T. Galloway,
*Chief of Bureau.*

Hon. James Wilson,
*Secretary of Agriculture.*

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# A BIOCHEMICAL STUDY OF THE CURLY-TOP OF SUGAR BEETS.

## INTRODUCTION.

The curly-top of sugar beets seems to have attracted notice first in California about 1898. It is characterized briefly by the following symptoms (Shaw, 1910):[1] An inward curling of the leaves, a distortion of the veins of the affected leaves, hairy roots, and checked growth. The menacing character of the disease has been pointed out in former publications of this Bureau (Townsend, 1908; Shaw, 1910), in which it has been shown to be responsible for great financial losses in the western beet districts, not only by stunting the growth of sugar beets but also by preventing the production of seed. The Office of Cotton and Truck Disease and Sugar-Plant Investigations has therefore undertaken a systematic study of this disease, looking toward the discovery of its cause and the means of prevention.

## CAUSE OF THE DISEASE.

Concerning the cause of this condition, a great variety of opinions have been expressed. Soon after its appearance in California the Spreckels Sugar Co. secured the opinion of a number of American and European experts, whose views have been summarized and discussed at length by Linhart (1901). They attributed the trouble to one of the following causes: Hot winds, insufficient fertilization, insects, micro-organisms, irregular irrigation and rainfall, and unknown atmospheric peculiarities. While later it was shown that curly-top develops after the bite of an insect (Ball, 1911), the curly-top leafhopper (*Eutettix tenella*), nothing is known concerning the mechanism by which this effect is produced or concerning the abnormal processes set up in the plant.

## OBJECT OF THE INVESTIGATION.

Mr. W. A. Orton, in charge of Sugar-Plant Investigations of the Bureau of Plant Industry, requested the writer to make a quantita-

[1] For complete references to the literature cited in the text by the author's name and the date of publication, see the list at the end of this bulletin.

tive study of the oxidizing enzyms of normal and curly-top beets. It was hoped that an extensive and accurate knowledge of the physiological behavior of healthy and unhealthy plants would throw light on the causes of the condition, as knowledge of these causes would bring the remedy for the trouble nearer.

## FORMER WORK ON RÔLE OF OXIDASES IN PLANT DISEASES.

The advisability of studying the oxidizing enzyms in connection with pathological conditions in plants is also suggested by the work of others. Mr. A. F. Woods (1902), formerly of this Bureau, explained the mosaic disease of tobacco on the basis of disturbances in the oxidase mechanism. He invariably found a greater quantity of oxidases in the spotted leaves than in the normal ones. He further proved that this excess of the oxidases seriously interfered with the action of diastases in the leaves and in this way brought about disturbances in the metabolism of the plant. This theory was criticized by Hunger (1903, 1905). Sorauer (1908) in studying the leaf-curl of potatoes came to the conclusion that the color changes accompanying the disease are not due to fusaria, other fungi, or bacteria, but to disturbances in the enzymotic equilibria. These disturbances manifest themselves in increased sugar and starch formation and therefore furnish a favorable medium for parasites. The latter, then, according to Sorauer, are responsible for the gross symptoms of the malady.

Sorauer's conclusions are based largely on experiments by Grüss. Sorauer emphasized principally the greater intensity of the peroxidase reaction and the diminution of the oxidase and tyrosinase reaction in the diseased tubers as compared with the healthy ones. Concordant views were expressed by Pozzi-Escot (1905), who attributed a number of plant diseases to excess of oxidases, inasmuch as the latter destroy the other enzyms taking part in metabolism. Doby (1911) recently made measurements of the oxidases in healthy and diseased potatoes and found no relationship between the oxidase content and the state of health of the tubers. Later, carrying out the experiments on a larger scale, Doby (1912) confirmed in general Sorauer's hypothesis concerning the enzymotic disturbances in the leaf-curl of potatoes. His results, moreover, show that in the diseased potatoes oxidases, peroxidases, and tyrosinases are present in increased quantities. The problem of the etiology of this disease has been discussed at length by Appel and Schlumberger (1911). A great deal more work must be done on the oxidases of normal and leaf-curled potatoes before any definite conclusions can be drawn. It is the intention of the writer to study this question when opportunity arises.

## RESULTS OBTAINED WITH GREENHOUSE MATERIAL IN 1911.

In the early spring of the year 1911 the oxidase contents of healthy and of diseased sugar beets grown in the greenhouse were compared. All of the plants were grown under nearly the same conditions. This became particularly evident among the healthy plants, which in size and appearance were very uniform indeed. The measurements were carried out on the leaves according to the method devised by the writer, the details of whose experiments are given in a previous bulletin (Bunzel, 1912). The results of those experiments are shown in concise form in Table I.

TABLE I.—*Oxidase content of healthy and diseased beets grown in the greenhouse.*

| Experiment No. | Juice used. | Manometer readings expressed in centimeters of mercury. |
|---|---|---|
| 1 | Juice of normal beet leaves | 1.16 |
| 2 | .....do | 1.07 |
| 3 | Juice of diseased beet leaves | 5.61 |
| 4 | .....do | 4.30 |
| 5 | Juice of normal beet leaves | 1.10 |
| 6 | .....do | 1.17 |
| 7 | Juice of diseased beet leaves | 2.72 |
| 8 | Juice of normal beet leaves | 1.19 |
| 9 | .....do | 1.21 |
| 10 | Juice of diseased beet leaves (showing slight symptoms only) | 1.51 |
| | Mean absorption in experiments with juice of normal plants (in terms of units,[1] 0.166). | 1.15 |
| | Mean absorption in experiments with juice of diseased plants (in terms of units,[1] 0.509). | 3.54 |

[1] The unit chosen was an oxidase solution of such strength that 1 liter of it could bring about the oxidation of the equivalent of 1 gram of hydrogen (Bunzel, 1912, p. 40).

The determinations given in Table I show a very striking difference between the juice of the normal and that of the diseased beet leaves. In all of the experiments the oxidase content, as indicated by the changes in pressure in the manometer resulting from oxygen absorption by the pyrogallol in the presence of the juice, is markedly greater in the diseased than in the healthy leaves. The oxidase content of the normal leaves seems to be fairly constant, while the juice of the curly-top beet leaves shows wide variations. The leaves used in experiment 3 give about five times as high a figure as normal leaves, while the leaves chosen in experiment 10 show an increase of only 25 per cent above the normal.

It is very interesting to note that the deviation in oxidase content of the pathological leaves, as measured by the method used, runs parallel with the appearance of the leaves. The plants used in experiment 3 showed very marked signs of curly-top, the leaves being small and shriveled and the hairy roots abundant, while the dis-

eased beet used in experiment 10, which showed a relatively low oxidase content, though higher than normal, had only a slight curling of the leaves.

## EXPERIMENTAL WORK.

Although these results are quite conclusive in showing the existence of differences in the oxidase mechanism of the healthy and diseased sugar beets grown in the greenhouse, it is not justifiable to apply these results to conditions in the field without further experimentation. Where sugar beets are grown on a commercial scale, an entirely different and widely varying environment prevails. A trip was therefore undertaken to Ogden, Utah, the center of an important district in Utah and southern Idaho, where about half a million tons of sugar beets are harvested every year. The writer spent the month of August, 1911, at the beet-sugar factory of the Amalgamated Sugar Co., where all of the experiments described in this paper were carried out. The main object of the experiments was to study variations in the concentration of oxidases in the fresh plants.

### SHORT DESCRIPTION OF APPARATUS USED IN THE FIELD.

As has been shown (Bunzel, 1912), a constant-temperature chamber is required for all experiments of this sort. The thermostat heretofore used is not well adapted to transportation, being large and difficult to set up. A special air thermostat, more durable and more easily installed, was therefore built for these experiments, differing from the former one principally in simplicity of construction. The heating was done by lamps mounted in a circle behind the fan, and at the front of the box was a swinging door instead of a sliding one, as in the other apparatus. The necessary switches, condensers, and dry cells were all mounted in a special compartment at one end of the thermostat, separated from the warm chamber. The thermostat, which has been slightly remodeled since being used for these experiments, will be described in detail and illustrated in a later publication.

### MATERIALS AND REAGENTS USED IN THE EXPERIMENTS.

The material used was obtained very largely from the gardens and fields of the temporary station of the Office of Sugar-Plant Investigations of the Bureau of Plant Industry at Ogden, Utah. The remainder was collected on various fields of sugar-beet farmers in that vicinity. Fresh material, collected within one or two hours, was used in all experiments. The method of preparing it has been described in a previous publication (Bunzel, 1912). After grinding the

plant in a meat chopper the juice was expressed through a piece of raw silk. In all of the experiments 8 c. c. of a 1 per cent solution of pyrogallol and 2 c. c. of the leaf juice were used with 1 c. c. of normal sodium hydroxid in the basket. The rate of shaking in all the experiments to be described was 5 excursions in 3.5 seconds, the magnitude of the excursions being 10 centimeters. The temperature during the experiments described was 40.5° C., remaining constant to 0.1 degree. In a number of cases samples of the ground material and of the juice were preserved for analysis.

## OXIDASE CONTENT OF LEAVES, ROOTS, AND SEEDS[1] OF NORMAL, HEALTHY PLANTS UNDER DIFFERENT CONDITIONS.

### Series 1, August 22, 1911.

Leaves of two types from normal, healthy beets were collected at 7 a. m. from the same patch of ground in Mr. Chandler's field—very large leaves 30 to 40 centimeters long and small ones less than 15 centimeters long. Oxidase apparatus Nos. 5 and 7 received the juice obtained from the small leaves, while Nos. 11 and 12 were used for the experiments with the large leaves.

Table II.—*Manometer readings obtained from juices of small and large leaves of healthy beets (series 1).*

| Time of reading manometer. | Time elapsed. | Temperature at time of measurement. | Manometer readings, expressed in centimeters of mercury. | | | |
|---|---|---|---|---|---|---|
| | | | Small leaves. | | Large leaves. | |
| | | | No. 5. | No. 7. | No. 11. | No. 12. |
| | *Minutes.* | °*C.* | | | | |
| 9.20 a. m. | 0 | 40.2 | 0 | 0 | 0 | 0 |
| 9.40 a. m. | 20 | 40.2 | −1.20 | −1.05 | − .40 | − .35 |
| 10.00 a. m. | 40 | 40.3 | −2.80 | −2.30 | −1.20 | − .80 |
| 10.20 a. m. | 60 | 40.2 | −3.35 | −2.80 | −1.30 | −1.00 |
| 10.40 a. m. | 80 | 40.2 | −3.40 | −3.00 | −1.30 | −1.00 |
| 11.00 a. m. | 100 | 40.2 | −3.60 | −3.15 | −1.45 | −1.10 |
| 11.20 a. m. | 120 | 40.2 | −3.60 | −3.20 | −1.50 | −1.10 |

| | |
|---|---|
| Mean for Nos. 5 and 7 | −3.40 |
| Mean for Nos. 11 and 12 | −1.30 |
| Activity of juice of small leaves (expressed in units, pyrogallol) | .499 |
| Activity of juice of large leaves (expressed in units, pyrogallol) | .184 |

The agreement between the duplicates in series 1 is fair, but the difference between the oxidase content of the small and large leaves is striking. The small leaves are nearly three times as rich in oxidases as the large ones.

[1] Throughout this paper "seed" is used in the usual sense of a group of calyxes, each containing several seeds.

SERIES 2, AUGUST 8, 1911.

The plants were very young, with leaves only 2 to 7 centimeters long, collected in the garden adjoining the station on the west. They had been protected by means of muslin nets against infestation by insects and presented the appearance of perfectly healthy plants.

TABLE III.—*Manometer readings obtained from juice of very young healthy beets (series 2).*

| Time of reading manometer | Time elapsed. | Manometer readings, expressed in centimeters of mercury, in apparatus— | |
|---|---|---|---|
| | | No. 3. | No. 5. |
| | *Minutes.* | | |
| 9.30 a. m. | 0 | 0 | 0 |
| 10.00 a. m. | 30 | − .75 | − .40 |
| 10.15 a. m. | 45 | − .95 | − .80 |
| 10.30 a. m. | 60 | −1.20 | −1.20 |
| 10.45 a. m. | 75 | −1.30 | −1.25 |
| 11.00 a. m. | 90 | −1.30 | −1.20 |

Activity of juice of leaves (expressed in units, pyrogallol) 0.180

Series 2 shows that the leaf juice of the healthy plants had an oxidase content approximately equal to that found for beets grown in the greenhouse. (Table I.) The mean result obtained in six separate experiments on greenhouse material was 0.166 (pyrogallol); in the carefully protected and necessarily healthy material of the field garden it was a little higher, 0.180 (pyrogallol). As shown below, the normal plants grown under natural conditions in the field had a tendency to yield slightly higher results than the normal greenhouse plants.

Results on the large leaves described in series 1 are in harmony with those given in Tables I and III, the oxidase content being 0.184 (pyrogallol). The small leaves collected from plants in the immediate vicinity of the large-leaved ones showed an oxidase content very much higher, i. e., 0.499 (pyrogallol). Although these small leaves showed no sign of disease, they were not strictly normal (?), inasmuch as their growth had been inhibited. Accompanying this stunted growth there was an increase in the oxidase content.

All of the plants examined were not collected at the same time of day. Series 3 to 7 were carried out to determine what variations, if any, took place in the concentration of the oxidases in the leaves at different periods of the day. These experiments make it possible to interpret correctly the differences observed between the plants living under different physiological conditions, even when the leaves were collected at different times of the same day.

SERIES 3 AND 4, AUGUST 25, 1911.

Large leaves, 30 centimeters long, of uniform size, were collected on the sugar-beet farm adjoining the sugar factory on the east. The leaves were picked from two plants of similar appearance, termed in this experiment *a* and *b*.

In series 3, the leaves were collected at sunrise (6.10 a. m.); and apparatus Nos. 3 and 7 received the juice of the leaves of plant *a*, while Nos. 11 and 12 were used for plant *b*.

In series 4, the leaves were collected at sundown (7.15 p. m.); and apparatus No. 3 was used for plant *a*, while Nos. 5 and 7 were used or plant *b*.

TABLE IV.—*Manometer readings obtained from juices of large beet leaves collected at different hours.*

SERIES 3.—LEAVES COLLECTED AT SUNRISE (6.10 A. M.).

| Time of reading manometer. | Time elapsed. | Manometer readings, expressed in centimeters of mercury. | | | |
|---|---|---|---|---|---|
| | | Plant *a*. | | Plant *b*. | |
| | | No. 3. | No. 7. | No. 11. | No. 12. |
| | *Minutes.* | | | | |
| 7.20 a. m. | 0 | 0 | 0 | 0 | 0 |
| 7.45 a. m. | 25 | −1.10 | − .80 | − .70 | − .75 |
| 8.10 a. m. | 50 | −1.60 | −1.30 | −1.30 | −1.40 |
| 8.30 a. m. | 70 | −1.90 | −1.70 | −1.50 | −1.80 |
| 8.50 a. m. | 90 | −1.90 | −1.70 | −1.75 | −1.80 |

SERIES 4.—LEAVES COLLECTED AT SUNDOWN (7.15 P. M.).

| | | | | *No. 5.* | *No. 7.* |
|---|---|---|---|---|---|
| 8.30 p. m. | 0 | 0 | ........ | 0 | 0 |
| 8.55 p. m. | 25 | −1.00 | ........ | − .90 | −1.10 |
| 9.20 p. m. | 50 | −2.20 | ........ | −2.00 | −2.00 |
| 9.40 p. m. | 70 | −2.90 | ........ | −2.70 | −2.60 |
| 10.00 p. m. | 90 | −3.30 | ........ | −3.00 | −2.70 |
| 10.20 p. m. | 110 | −3.30 | ........ | −3.20 | −3.10 |

*Series 3.*—Activity of juice of leaves *a* (expressed in units, pyrogallol) ........ 0.259
Activity of juice of leaves *b* (expressed in units, pyrogallol) ........ .256
*Series 4.*—Activity of juice of leaves *a* (expressed in units, pyrogallol) ........ .468
Activity of juice of leaves *b* (expressed in units, pyrogallol) ........ .453

SERIES 5, 6, AND 7, AUGUST 30, 1911.

Five large leaves, 25 centimeters long, from each of two plants (*a* and *b*) of similar appearance were collected in the field adjoining the factory on the east, shortly before sunrise (6 a. m.) in series 5, at 9.30 a. m. in series 6, and at 2.45 p. m. in series 7. Apparatus Nos. 3 and 5 received the juice of the leaves of plant *a;* Nos. 6 and 7 the juice of the leaves of plant *b*.

TABLE V.—*Manometer readings obtained from juices of large beet leaves collected at different hours.*

SERIES 5.—LEAVES COLLECTED AT 6 A. M.

| Time of reading manometer. | Time elapsed. | Manometer readings, expressed in centimeters of mercury. | | | |
|---|---|---|---|---|---|
| | | Plant *a*. | | Plant *b*. | |
| | | No. 3. | No. 5. | No. 6. | No. 7. |
| | *Minutes.* | | | | |
| 7.00 a. m | 0 | 0 | 0 | 0 | 0 |
| 7.15 a. m | 15 | −.60 | −.70 | −.65 | −.70 |
| 7.30 a. m | 30 | −1.05 | −1.00 | −1.05 | −1.20 |
| 7.45 a. m | 45 | −1.20 | −1.15 | −1.20 | −1.35 |
| 8.00 a. m | 60 | −1.40 | −1.50 | −1.40 | −1.60 |
| 8.15 a. m | 75 | −1.55 | −1.60 | −1.60 | −1.70 |
| 8.30 a. m | 90 | −1.75 | −1.70 | −1.80 | −1.90 |
| 8.45 a. m | 105 | −1.80 | −1.75 | −1.80 | −1.90 |

SERIES 6.—LEAVES COLLECTED AT 9.30 A. M.

| | | | | | |
|---|---|---|---|---|---|
| 10.00 a. m | 0 | 0 | 0 | 0 | 0 |
| 10.20 a. m | 20 | −.80 | −.70 | −.75 | −.75 |
| 10.40 a. m | 40 | −1.20 | −1.30 | −1.35 | −1.30 |
| 11.00 a. m | 60 | −1.50 | −1.60 | −1.50 | −1.50 |
| 11.20 a. m | 80 | −1.65 | −1.70 | −1.70 | −1.60 |
| 11.40 a. m | 100 | −1.80 | −1.85 | −1.85 | −1.80 |
| 12.00 m | 120 | −1.80 | −1.80 | −1.90 | −1.80 |

SERIES 7.—LEAVES COLLECTED AT 2.45 P. M.

| | | | | | |
|---|---|---|---|---|---|
| 3.35 p. m | 0 | 0 | 0 | 0 | 0 |
| 3.55 p. m | 20 | −.80 | −.80 | −.80 | −.70 |
| 4.15 p. m | 40 | −1.40 | −1.70 | −1.30 | −1.30 |
| 4.35 p. m | 60 | −1.90 | −1.90 | −1.80 | −1.60 |
| 4.55 p. m | 80 | −2.15 | −2.00 | −2.00 | −1.90 |
| 5.15 p. m | 100 | −2.25 | −2.15 | −2.10 | −1.90 |
| 5.25 p. m | 110 | −2.25 | −2.20 | −2.10 | −1.90 |

*Series 5.*—Activity of juice of leaves *a* (expressed in units, pyrogallol) 0.256
Activity of juice of leaves *b* (expressed in units, pyrogallol) .266
*Series 6.*—Activity of juice of leaves *a* (expressed in units, pyrogallol) .259
Activity of juice of leaves *b* (expressed in units, pyrogallol) .266
*Series 7.*—Activity of juice of leaves *a* (expressed in units, pyrogallol) .319
Activity of juice of leaves *b* (expressed in units, pyrogallol) .288

As series 3 to 7 show, there is a marked and gradual rise in the concentration of the oxidases in the leaves of the same plant as the time of the day proceeds. As indicated by the determination of the solids and ash of the same samples (shown in Table XIX), there is a distinct parallelism between the quantity of oxidase and of solids in the samples. The atmosphere during the month of August, 1911, at and in the vicinity of Ogden was abnormally dry and hot, so that the intense transpiration on the part of the plant is easily explained.

Incidentally three experiments (series 8 to 10) were made to study the distribution of the oxidases in the beets.

SERIES 8, AUGUST 23, 1911.

An apparently healthy seed plant, 1.5 meters high, was collected at 7.30 a. m. in the garden adjoining the station on the east. This plant was fresh, green, and in excellent condition. Apparatus No. 3 received the juice of the root, No. 7 the juice of the midribs of leaves and pedicels, No. 11 the juice of the stem, No. 12 the seed juice, and No. 13 the leaf juice.

TABLE VI.—*Manometer readings obtained from juices of different parts of a single plant (series 8).*

| Time of reading manometer. | Time elapsed. | Manometer readings, expressed in centimeters of mercury, in apparatus— | | | | |
|---|---|---|---|---|---|---|
| | | No. 3 (root). | No. 7 (midribs and pedicels). | No. 11 (stem). | No. 12 (seeds). | No. 13 (leaves). |
| | *Minutes.* | | | | | |
| 9.35 a. m. | 0 | 0 | 0 | 0 | 0 | 0 |
| 10.00 a. m. | 25 | − .65 | −1.10 | 0 | −1.80 | − .80 |
| 10.20 a. m. | 45 | −1.20 | −1.30 | 0 | −3.00 | −1.50 |
| 10.40 a. m. | 65 | −1.20 | −1.60 | 0 | −4.00 | −2.00 |
| 11.00 a. m. | 85 | −1.40 | −1.75 | − .10 | −4.70 | −2.40 |
| 11.20 a. m. | 105 | −1.35 | −1.70 | − .10 | −4.50 | −2.50 |

Activity of juice of roots (expressed in units, pyrogallol) ........ 0.194
Activity of juice of midribs of leaves and pedicels (expressed in units, pyrogallol) ........ .245
Activity of juice of stem (expressed in units, pyrogallol) ........ .014
Activity of juice of seeds (expressed in units, pyrogallol) ........ .648
Activity of juice of leaves (expressed in units, pyrogallol) ........ .360

SERIES 9, AUGUST 26, 1911.

A healthy looking, seed-carrying plant grown under the same conditions as that in series 8 was used. It was cut at 7.30 a. m. Apparatus No. 3 was used for the leaves, No. 5 for the seeds, No. 7 for the upper third of the root, and No. 11 for the lower third of the root.

TABLE VII.—*Manometer readings obtained from juices of different parts of a single plant (series 9).*

| Time of reading manometer. | Time elapsed. | Manometer readings, expressed in centimeters of mercury, in apparatus— | | | |
|---|---|---|---|---|---|
| | | No. 3 (leaves). | No. 5 (seeds). | No. 7 (upper third of root). | No. 11 (lower third of root). |
| | *Minutes.* | | | | |
| 9.40 a. m. | 0 | 0 | 0 | 0 | 0 |
| 10.00 a. m. | 20 | −1.05 | −2.00 | −1.40 | − .85 |
| 10.20 a. m. | 40 | −1.60 | −3.50 | −2.20 | −1.80 |
| 10.40 a. m. | 60 | −1.80 | −4.00 | −2.20 | −2.40 |
| 11.00 a. m. | 80 | −2.00 | −4.10 | −2.40 | −2.70 |
| 11.20 a. m. | 100 | −2.00 | −4.05 | −2.30 | −2.65 |

Activity of juice of leaves (expressed in units, pyrogallol) ........ 0.288
Activity of juice of seeds (expressed in units, pyrogallol) ........ .583
Activity of juice of upper third of root (expressed in units, pyrogallol) ........ .331
Activity of lower third of root (expressed in units, pyrogallol) ........ .381

SERIES 10, AUGUST 18, 1911.

The plant used was of the same type as that used in series 8 and 9. It was collected at 9 a. m. Apparatus No. 3 was used for leaves, No. 5 for seeds, No. 6 for pedicels and midribs of leaves, No. 11 for stems, No. 12 for the upper half of the root, and No. 13 for the lower half of the root.

TABLE VIII.—*Manometer readings obtained from juices of different parts of a single plant (series 10).*

| Time of reading manometer. | Time elapsed. | Manometer readings, expressed in centimeters of mercury, in apparatus— | | | | | |
|---|---|---|---|---|---|---|---|
| | | No. 3 (leaves). | No. 5 (seeds). | No. 6 (pedicels and midribs). | No. 11 (stems). | No. 12 (upper half of root). | No. 13 (lower half of root). |
| | *Minutes.* | | | | | | |
| 10.30 a. m. | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 11.00 a. m. | 30 | −1.20 | −2.80 | −1.00 | − .10 | − .90 | −1.10 |
| 11.20 a. m. | 50 | −1.55 | −3.70 | −1.15 | − .15 | −1.05 | −1.35 |
| 11.40 a. m. | 70 | −1.60 | −4.05 | −1.10 | − .20 | −1.00 | −1.60 |
| 12.00 m. | 90 | −1.60 | −4.10 | −1.20 | − .15 | −1.00 | −1.65 |

| | |
|---|---|
| Activity of juice of leaves (expressed in units, pyrogallol) | 0.230 |
| Activity of juice of seeds (expressed in units, pyrogallol) | .590 |
| Activity of juice of midribs and pedicels (expressed in units, pyrogallol) | .173 |
| Activity of juice of stems (expressed in units, pyrogallol) | .022 |
| Activity of juice of upper half of roots (expressed in units, pyrogallol) | .144 |
| Activity of juice of lower half of roots (expressed in units, pyrogallol) | .237 |

According to the last three experiments (series 8–10), there is a great difference in the oxidase content of different parts of the same plant. In the chlorophyll-containing portions of the sugar-beet plant the activity shows a parallelism with the intensity of the color. The seeds which are greenest generally lead. The oxidase content of their juice in these experiments ranges from 0.58 to 0.65 units (pyrogallol). The juice of the seeds, it is interesting to note, is darker than the juice of the other portions of the plant. The leaves follow with an oxidase content of 0.23 to 0.36 units (pyrogallol); the stem as well as its juice presents a pale green appearance and yields a value of 0.014 to 0.022 units (pyrogallol), while the somewhat darker pedicels and midribs have a juice with a higher oxidase content, i. e., of 0.17 to 0.25 units (pyrogallol). The results obtained on the roots confirm those obtained in earlier experiments. The lower part of the root gives a more active juice than the upper. The difference is not always marked, but in some cases (series 14) it becomes almost as 2 to 1. The activity of the juice of the upper part of the root is generally below that of normal leaves, but in some cases (series 9) somewhat exceeds it. The activity of the juice of the lower part of the root generally exceeds that of normal leaves

(series 9, 10, 14, 15, 16). In the plants where growth has been stunted for some reason, the roots show no marked anomaly of oxidase content; therefore the leaves always give a very much higher figure, as will be seen later. Series 11 was carried out to make certain that the very high oxidase content obtained for the seeds was not merely accidental.

SERIES 11, AUGUST 9, 1911.

Seeds were collected at 7.30 a. m. from a tall, healthy plant in the garden adjoining the station on the east. Six experiments were carried out.

TABLE IX.—*Manometer readings obtained from juices of beet seeds (series 11).*

| Time of reading manometer. | Time elapsed. | Manometer readings, expressed in centimeters of mercury, in apparatus— | | | | | |
|---|---|---|---|---|---|---|---|
| | | No. 3. | No. 5. | No. 7. | No. 11. | No. 12. | No. 13. |
| | *Minutes.* | | | | | | |
| 10.30 a. m. | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 11.00 a. m. | 30 | −2.30 | −2.00 | −2.45 | −1.90 | −2.25 | −2.30 |
| 11.20 a. m. | 50 | −3.20 | −3.10 | −3.40 | −3.10 | −3.10 | −3.15 |
| 11.40 a. m. | 70 | −4.00 | −3.90 | −4.10 | −4.00 | −4.05 | −4.20 |
| 12.00 m. | 90 | −4.20 | −4.00 | −4.10 | −4.05 | −4.00 | −4.15 |

Mean reading obtained ........ 4.08
Activity of juice of seeds (expressed in units, pyrogallol) ........ .587

The results of this series also show the high oxidase content of the seeds as compared with the other parts of the plant.

## OXIDASE CONTENT OF LEAVES AND ROOTS OF DISEASED PLANTS.

The relations in normal, healthy plants having been elucidated, a study of the oxidase contents of the leaves and roots of the diseased plants was then undertaken.

SERIES 12, AUGUST 22, 1911.

Curly-top beets and controls were collected on the large Government field, Wilson Lane, Utah, at 1 p. m. All the leaves, except the extreme outside ones, were affected. These outside leaves were quite large (25 to 30 centimeters long), while the infected inner leaves measured from 10 to 20 centimeters. Apparatus Nos. 4 and 5 received the juices of the insect-injured leaves, Nos. 7 and 12 were used for the healthy ones, while in Nos. 13 and 15 experiments were made with the juice obtained from the root.

TABLE X.—*Manometer readings obtained from juices of diseased leaves, healthy leaves, and roots (series 12).*

| Time of reading manometer. | Time elapsed. | Temperature at time of measurement. | Manometer readings, expressed in centimeters of mercury. | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Diseased leaves. | | Healthy leaves. | | Roots. | |
| | | | No. 4. | No. 5. | No. 7. | No. 12. | No. 13. | No. 15. |
| | *Minutes.* | °*C.* | | | | | | |
| 3.05 p. m. | 0 | 40.0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3.25 p. m. | 20 | 40.1 | −1.10 | −1.20 | −.60 | −.30 | Lost. | −.25 |
| 3.45 p. m. | 40 | 40.1 | −2.25 | −2.35 | −.90 | −.60 | (1) | −1.00 |
| 4.05 p. m. | 60 | ........ | −2.70 | −2.60 | −1.10 | −.80 | ........ | −1.30 |
| 4.25 p. m. | 80 | ........ | −2.70 | −2.70 | −1.10 | −.95 | ........ | −1.30 |

[1] Stopcock opened.

Activity of juice of curly-top leaves (expressed in units, pyrogallol) ........ 0.388
Activity of juice of large, healthy control leaves (expressed in units, pyrogallol) ........ .144
Activity of juice of curly-top beets (expressed in units, pyrogallol) ........ .187

Series 12 shows the great divergence in the oxidase content of healthy and diseased leaves growing on the same plant. The small, curly-top leaves were nearly three times as active as the large normal ones. There seems to be no marked difference between the oxidase content of the roots of the healthy and of the curly-top plants, as is seen by comparing the figures of this experiment with those of series 8, 9, 10, and 13. The divergence between the oxidase content of the healthy and normal leaves and those whose growth has been stunted is not due simply to a difference in size, inasmuch as small but perfectly healthy leaves, as used in series 2, have a low oxidase content.

SERIES 13, AUGUST 21, 1911.

Curly-top plants with pronounced symptoms were collected at 7 a. m. from the large field of the temporary field station. The large outer leaves, which were apparently normal, were separated. Apparatus Nos. 5 and 11 received the normal, while Nos. 7 and 12 received the affected leaf juices. The activity of the roots was studied in Nos. 13 and 15.

TABLE XI.—*Manometer readings obtained from juices of normal leaves, diseased leaves, and roots (series 13).*

| Time of reading manometer. | Time elapsed. | Temperature at time of measurement. | Manometer readings, expressed in centimeters of mercury. | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Normal leaves. | | Diseased leaves. | | Roots. | |
| | | | No. 5. | No. 11. | No. 7. | No. 12. | No. 13. | No. 15. |
| | *Minutes.* | °*C.* | | | | | | |
| 10.30 a. m. | 0 | 40.0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 11.00 a. m. | 30 | 40.1 | −.50 | −.70 | −1.40 | −1.00 | −1.20 | −.50 |
| 11.45 a. m. | 75 | 40.1 | −1.05 | −1.40 | −2.30 | −2.40 | −1.90 | −1.30 |
| 12.00 m. | 90 | 40.1 | −1.20 | −1.35 | −2.50 | −2.70 | −2.20 | −1.50 |
| 12.15 p. m. | 105 | 40.1 | −1.30 | −1.35 | −2.50 | −2.80 | −2.00 | −1.50 |

Activity of juice of healthy leaves (expressed in units, pyrogallol) ........ 0.191
Activity of juice of curly-top leaves (expressed in units, pyrogallol) ........ .381
Activity of juice of roots (expressed in units, pyrogallol) ........ .252

SERIES 14, AUGUST 24, 1911.

Stunted plants and large controls were collected on Mr. Chandler's farm at 1 p. m. No explanation could be found for the stunted growth of the small plants, which occurred isolated in the midst of a group of plants four to six times their size. In this experiment the upper halves of the roots were ground to pulp separately from the lower halves. No. 3 gives the activity of the leaves of the small plant, No. 5 the upper half of the root of the same plant, No. 7 the lower half of the root of the same plant, No. 11 the leaves of the large control plant, No. 12 the upper half of the root of the same plant, No. 13 the lower half of the root of the same plant.

TABLE XII.—*Manometer readings obtained from juices of stunted beets and well-grown beets (series 14).*

| Time of reading manometer. | Time elapsed. | Temperature at time of measurement. | Manometer readings, expressed in centimeters of mercury. | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Small, stunted plants. | | | Large control plants. | | |
| | | | No. 3 (leaves). | No. 5 (upper half of root). | No. 7 (lower half of root). | No. 11 (leaves). | No. 12 (upper half of root). | No. 13 (lower half of root). |
| | *Minutes.* | °*C.* | | | | | | |
| 2.55 p. m. | 0 | 40.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3.25 p. m. | 30 | 40.1 | −1.50 | − .60 | − .75 | − .90 | − .80 | − .70 |
| 3.45 p. m. | 50 | 40.1 | −2.20 | − .70 | −1.00 | −1.00 | −1.10 | −1.50 |
| 4.05 p. m. | 70 | 40.1 | −2.40 | − .60 | −1.10 | −1.40 | −1.10 | −1.70 |
| 4.25 p. m. | 90 | 40.1 | −2.50 | − .60 | −1.10 | −1.40 | −1.20 | −2.00 |
| 4.45 p. m. | 110 | 40.1 | −2.55 | − .60 | −1.10 | −1.40 | −1.20 | −2.00 |

| | |
|---|---|
| Activity of juice of leaves of small plants (expressed in units, pyrogallol) | 0.367 |
| Activity of juice of upper half of root (expressed in units, pyrogallol) | .086 |
| Activity of juice of lower half of root (expressed in units, pyrogallol) | .158 |
| Activity of juice of leaves of large control plants (expressed in units, pyrogallol) | .201 |
| Activity of juice of upper half of root (expressed in units, pyrogallol) | .172 |
| Activity of juice of lower half of root (expressed in units, pyrogallol) | .288 |

The results given in Table XII show that the leaves of plants which are small without showing signs of the curly-top disease are richer in oxidases than the leaves of large healthy plants growing in their immediate vicinity. The lower half of the roots of both kinds is richer in oxidases than the upper half.

SERIES 15, AUGUST 25, 1911.

Stunted plants and large controls were collected on Mr. Chandler's farm at 9 a. m. The growth of small plants had been retarded by drought. The roots were cut into three parts of equal length and only the upper and lower thirds used in the experiment. Apparatus No. 3 received the leaf juice of the small plants, No. 5 the juice of the upper third of the small roots, No. 7 the lower third of the small roots, No. 11 the leaf juice of the large control plants, No. 12 the upper third of large roots, and No. 13 the lower third of the large roots.

TABLE XIII.—*Manometer readings obtained from juices of stunted beets and well-grown beets (series 15).*

| Time of reading manometer. | Time elapsed. | Temperature at time of measurement. | Manometer readings, expressed in centimeters of mercury. | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Small, stunted plants. | | | Large control plants. | | |
| | | | No. 3 (leaves). | No. 5 (upper third of root). | No. 7 (lower third of root). | No. 11 (leaves). | No. 12 (upper third of root). | No. 13 (lower third of root). |
| | *Minutes.* | *° C.* | | | | | | |
| 10.30 a. m. | 0 | 40.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10.50 a. m. | 20 | 40.1 | − .90 | − .20 | −1.50 | − .40 | − .80 | − .60 |
| 11.10 a. m. | 40 | 40.2 | −1.50 | − .50 | −2.30 | − .80 | −1.10 | −1.30 |
| 11.30 a. m. | 60 | 40.2 | −1.60 | − .90 | −2.50 | − .75 | −1.15 | −1.70 |
| 11.45 a. m. | 75 | 40.2 | −1.60 | − .80 | −2.90 | −1.00 | −1.20 | −2.40 |
| 12.00 m. | 90 | 40.2 | −1.60 | − .90 | −2.80 | −1.00 | −1.20 | −2.50 |

Activity of juice of leaves of small plants (expressed in units, pyrogallol) ........ 0.230
Activity of juice of upper third of root (expressed in units, pyrogallol) ........ .130
Activity of juice of lower third of root (expressed in units, pyrogallol) ........ .403
Activity of juice of leaves of large control plants (expressed in units, pyrogallol) ........ .143
Activity of juice of upper third of root (expressed in units, pyrogallol) ........ .170
Activity of juice of lower third of root (expressed in units, pyrogallol) ........ .364

While there is no striking difference between the roots of the stunted and the normal plants, there is again a marked difference between the leaves of the two kinds of plants. The small leaves in this experiment show an increased oxidase content as compared with the normal leaves. In agreement with the preceding experiment, the lower portions of the roots are more active than the upper portions. To get further evidence on this point, series 16 and 17 were carried out.

SERIES 16, AUGUST 25, 1911.

Curly-top beets were collected on the large field of the station at 2 p. m. Both leaves and beets were very small, the leaves ranging from 4 to 12 centimeters in length, while the beets varied in weight from 100 to 200 grams each. Apparatus Nos. 3 and 5 received the juice of the leaves, Nos. 7 and 11 the juice of the upper third of the roots, and Nos. 12 and 13 that of the lower third.

TABLE XIV.—*Manometer readings obtained from juices of small diseased beets (series 16).*

| Time of reading manometer. | Time elapsed. | Temperature at time of measurement. | Manometer readings, expressed in centimeters of mercury. | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Leaves. | | Upper third of roots. | | Lower third of roots. | |
| | | | No. 3. | No. 5. | No. 7. | No. 11. | No. 12. | No. 13. |
| | *Minutes.* | *° C.* | | | | | | |
| 3.20 p. m. | 0 | 40.2 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3.40 p. m. | 20 | 40.2 | −1.30 | −1.15 | −1.20 | − .50 | −1.00 | −0.80 |
| 4.00 p. m. | 40 | 40.2 | −1.80 | −1.60 | −1.60 | −1.00 | −1.80 | −1.60 |
| 4.20 p. m. | 60 | 40.2 | −1.90 | −1.80 | −1.80 | −1.20 | −2.10 | −2.10 |
| 4.40 p. m. | 80 | 40.2 | −2.00 | −1.90 | −1.90 | −1.40 | −2.50 | −2.60 |
| 5.00 p. m. | 100 | 40.1 | −2.05 | −1.90 | −1.90 | −1.70 | −3.20 | −3.20 |
| 5.15 p. m. | 115 | 40.1 | −2.10 | −1.95 | −2.00 | −1.60 | −3.30 | −3.35 |

Activity of juice of leaves (expressed in units, pyrogallol) ........ 0.288
Activity of juice of upper third of roots (expressed in units, pyrogallol) ........ .259
Activity of juice of lower third of roots (expressed in units, pyrogallol) ........ .475

SERIES 17, AUGUST 26, 1911.

Curly-top beets were collected on one large field of the station at 1 p. m. Apparatus Nos. 3 and 5 received the juice of the lower fifth of the roots and Nos. 7 and 12 the juice of the top fifth.

TABLE XV.—*Manometer readings obtained from juices of diseased beet roots (series 17).*

| Time of reading manometer. | Time elapsed. | Temperature at time of measurement. | Manometer readings, expressed in centimeters of mercury. | | | |
|---|---|---|---|---|---|---|
| | | | Lowest fifth of roots. | | Top fifth of roots. | |
| | | | No. 3. | No. 5. | No. 7. | No. 12. |
| | *Minutes.* | °*C.* | | | | |
| 3.00 p. m | 0 | 40.1 | 0 | 0 | 0 | 0 |
| 3.20 p. m | 20 | 40.2 | − .55 | − .80 | − .70 | − .60 |
| 3.40 p. m | 40 | 40.2 | −1.50 | −1.50 | −1.50 | −1.30 |
| 4.00 p. m | 60 | 40.2 | −2.30 | −2.30 | −2.10 | −1.60 |
| 4.20 p. m | 80 | 40.2 | −2.75 | −2.70 | −2.30 | −2.00 |
| 4.40 p. m | 100 | 40.2 | −3.40 | −3.30 | −2.30 | −2.00 |
| 5.00 p. m | 120 | 40.2 | −3.45 | −3.50 | −2.40 | −2.00 |

Activity of juice of lowest fifth of the roots (expressed in units, pyrogallol) ........ 0.504
Activity of the juice of top fifth of the roots (expressed in units, pyrogallol) ........ .317

Series 17, with the observations previously cited, shows that the concentration of the oxidases in the lower portion of the root is two to three times greater than in the upper portion.

SERIES 18 AND 19, AUGUST 3 AND 4, 1911.

Roots of curly-top plants that had been siloed over the previous winter were used in these experiments. The plants showed no symptoms in the season of the year 1910. Those used belonged to the type called "trotzer," i. e., they failed to develop a seed stem and failed entirely to flower. They also showed marked curling of the leaves. The plants were collected in the garden adjoining the field station on the east at 8 a. m.—on August 3 for series 18 and on August 4 for series 19. Apparatus Nos. 3 and 5 contained the juice of the leaves, Nos. 7 and 11 the juice of the roots.

TABLE XVI.—*Manometer readings obtained from juices of curly-top beet leaves and roots.*

SERIES 18.—COLLECTED AT 8 A. M. ON AUGUST 3.

| Time of reading manometer. | Time elapsed. | Manometer readings, expressed in centimeters of mercury. | | | |
|---|---|---|---|---|---|
| | | Leaves. | | Roots. | |
| | | No. 3. | No. 5. | No. 7. | No. 11. |
| | *Minutes.* | | | | |
| 10.00 a. m | 0 | 0 | 0 | 0 | 0 |
| 10.30 a. m | 30 | −1.10 | − .80 | − .40 | − .65 |
| 11.30 a. m | 90 | −2.10 | −1.90 | − .80 | − .95 |
| 11.50 a. m | 110 | −2.80 | −2.70 | −1.20 | −1.35 |
| 12.10 p. m | 130 | −3.40 | −3.55 | −1.50 | −1.55 |
| 12.20 p. m | 140 | −3.35 | −3.55 | −1.50 | −1.45 |

SERIES 19.—COLLECTED AT 8 A. M. ON AUGUST 4.

| Time of reading manometer. | Time elapsed. | No. 3. | No. 5. | No. 7. | No. 11. |
|---|---|---|---|---|---|
| | *Minutes.* | | | | |
| 10.30 a. m | 0 | 0 | 0 | 0 | 0 |
| 10.45 a. m | 15 | −1.25 | −1.00 | − .50 | − .60 |
| 11.00 a. m | 30 | −1.65 | −1.80 | − .70 | − .65 |
| 11.15 a. m | 45 | −2.10 | −2.35 | −1.00 | − .90 |
| 11.25 a. m | 55 | −2.90 | −2.80 | −1.20 | −1.10 |
| 11.45 a. m | 75 | −3.10 | −3.10 | −1.30 | −1.35 |
| 12.00 m | 90 | −3.05 | −3.15 | −1.35 | −1.20 |

*Series 18.*—Activity of juice of leaves (expressed in units, pyrogallol) ........ 0.496
Activity of juice of roots (expressed in units, pyrogallol) ........ .216
*Series 19.*—Activity of juice of leaves (expressed in units, pyrogallol) ........ .446
Activity of juice of roots (expressed in units, pyrogallol) ........ .183

According to the results in both series 18 and 19 the leaves of the curly-top plants again show a higher oxidase content than was noted in connection with the healthy plants.

SERIES 20, AUGUST 5, 1911.

Curly-top plants collected at 7.30 a. m. in the garden adjoining the field station on the east were used. The roots of these plants had been siloed over winter and showed no symptoms of curly-top in the season of 1910. The plants had well-developed seed stems and carried seed. Apparatus Nos. 3 and 5 received the juice of the leaves, Nos. 7 and 12 that of the roots.

TABLE XVII.—*Manometer readings obtained from juices of curly-top beet leaves and roots (series 20).*

| Time of reading manometer. | Time elapsed. | Manometer readings, expressed in centimeters of mercury. | | | |
|---|---|---|---|---|---|
| | | Leaves. | | Roots. | |
| | | No. 3. | No. 5. | No. 7. | No. 12. |
| | *Minutes.* | | | | |
| 10.45 a. m | 0 | 0 | 0 | 0 | 0 |
| 11.10 a. m | 25 | −1.35 | −1.50 | − .80 | − .60 |
| 11.30 a. m | 45 | −2.10 | −2.00 | −1.30 | −1.80 |
| 11.50 a. m | 65 | −2.80 | −2.75 | −1.70 | −2.00 |
| 12.10 p. m | 85 | −2.85 | −2.70 | −2.20 | −2.10 |
| 12.25 p. m | 100 | −2.90 | −2.70 | −2.20 | −2.30 |

Activity of juice of leaves (expressed in units, pyrogallol) ........ 0.403
Activity of juice of roots (expressed in units, pyrogallol) ........ .324

Series 20 also shows that in curly-top seed-carrying sugar-beet plants the oxidase content is high. It seemed interesting to determine the oxidase content of plants showing the "trotzer" condition, but healthy and normal in other respects. Series 21 bears on this point.

SERIES 21, AUGUST 7, 1911.

The plants were "trotzer" beets, normal in other respects. They were collected on the eastern side of the field station at 12.30 p. m. Apparatus Nos. 3 and 5 received the juice of the leaves, Nos. 7 and 11 that of the roots.

TABLE XVIII.—*Manometer readings obtained from normal "trotzer" beets (series 21).*

| Time of reading manometer. | Time elapsed. | Manometer readings, expressed in centimeters of mercury. | | | |
|---|---|---|---|---|---|
| | | Leaves. | | Roots. | |
| | | No. 3. | No. 5. | No. 7. | No. 11. |
| | *Minutes.* | | | | |
| 2.30 p. m. | 0 | 0 | 0 | 0 | 0 |
| 3.00 p. m. | 30 | −1.60 | −1.25 | −1.00 | −1.10 |
| 3.20 p. m. | 50 | −2.30 | −2.10 | −1.60 | −1.50 |
| 3.40 p. m. | 70 | −3.00 | −3.10 | −1.50 | −1.55 |
| 4.00 p. m. | 90 | −3.15 | −3.05 | −1.70 | −1.60 |

Activity of juice of leaves (expressed in units, pyrogallol) ........ 0.446
Activity of juice of roots (expressed in units, pyrogallol) ........ .237

It appears then that the leaves of the plants which failed to develop seed, the so-called "trotzer" beets, also have an increased oxidase content. The increased oxidase content of the plants whose growth has been retarded is not simply due to the lack of development, but rather to inadequate development. The plants used in series 2 were smaller than those used in any one of the previous experiments, but they were small for natural reasons; there was every reason to believe that they would ultimately develop into large and healthy plants. The oxidase content of their leaves is approximately that found in the leaves of normal plants grown in the greenhouse or nearly that of healthy plants grown in the field.

The plants which were small because of retarded growth, on the other hand, show entirely different behavior. Their leaves have an oxidizing power two to three times as great as that of the normal plants. This is the case whether the retardation of growth is due to curly-top, to drought, to dwarfing from excessive irrigation, or whether the retardation is only a partial one, such as the failure to develop seeds and stems on the part of biennially grown sugar beets. It is therefore evident that the oxidase content is not a function of the size of the leaves, since small, normal young leaves have no more oxidase than larger, older normal ones. Small, stunted leaves, on the other hand, have an increased oxidase content independent of their size.

TABLE XIX.—*Determination of solids, ash, and nitrogen in the samples of sugar beets collected.*

| Sample No. | Condition. | Part of plant used. | Shape. | Place of collection. | Day of August. | Hour. | Solids in fresh material: Soluble in alcohol. | Solids in fresh material: Insoluble in alcohol. | Solids in fresh material: Total. | Ash: In alcohol-soluble material, all-solids basis. | Ash: In alcohol-insoluble material: Dried. | Ash: In alcohol-insoluble material: All-solids basis. | Ash: Total, all-solids basis. | Nitrogen: In alcohol-insoluble material. | Nitrogen: All-solids basis. | Cane sugar, all-solids basis. | Oxidases: Green material. | Oxidases: $\frac{R \times 100}{J}$. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | B | C | D | E | F | G | H | I | J | K | L | M | N | O | P | Q | R | S |
| | | | | | | | *P. ct.* | *P. ct.* | *P. ct.* | *P. ct.* | *P. ct.* | *P. ct.* | *P. ct.* | *P. ct.* | *P. ct.* | *P. ct.* | *Units.* | |
| 1 | Curly-top; showed no symptoms last year; siloed over winter; "trotzer." | Leaves | Whole | Garden east of station. | 3 | 8.00 a. m. | 5.10 | 11.24 | 16.34 | 1.28 | 18.69 | 12.85 | 14.13 | 5.36 | 3.69 | 1.84 | 0.496 | 3.02 |
| 2 | do | Roots | Cubes, 1 cm. | do | 3 | 8.00 a. m. | 8.10 | 6.41 | 14.51 | .895 | 8.85 | 3.90 | 4.80 | .76 | .335 | 37.40 | .216 | 1.49 |
| 3 | do | Leaves | Whole | do | 4 | 8.00 a. m. | 4.10 | 11.04 | 15.14 | 2.18 | 21.20 | 15.46 | 17.64 | 4.91 | 3.58 | 1.19 | .446 | 2.94 |
| 4 | do | Roots | Cubes, 1 cm. | do | 4 | 8.00 a. m. | 10.05 | 6.48 | 16.53 | 1.09 | 7.96 | 3.12 | 4.21 | .90 | .352 | 48.10 | .183 | 1.11 |
| 5 | do | Leaves | Whole | do | 5 | 7.30 a. m. | 3.90 | 12.64 | 16.54 | 2.18 | 35.53 | 27.18 | 29.36 | 2.95 | 2.25 | .41 | .403 | 2.44 |
| 6 | do | Roots | Pulp | do | 5 | 7.30 a. m. | 9.40 | 6.56 | 15.96 | .50 | 10.54 | 4.33 | 4.83 | .83 | .341 | 47.50 | | |
| 7 | do | do | Juice | do | 5 | 7.30 a. m. | | 1.06 | | | 45.32 | | | | | | .324 | |
| 8 | Apparently normal; siloed over winter; "trotzer." | Leaves | Pulp | do | 7 | 12.30 p. m. | 3.80 | 14.47 | 18.27 | 3.04 | 24.04 | 19.08 | 22.12 | 4.27 | 3.38 | .77 | | |
| 9 | do | Roots | do | do | 7 | 12.30 p. m. | 9.20 | 2.09 | 11.29 | 1.33 | 11.09 | 2.06 | 3.39 | 1.07 | .20 | 68.20 | | |
| 10 | do | Leaves | Juice | do | 7 | 12.30 p. m. | | 8.74 | | | 32.38 | | | 5.60 | | | .446 | |
| 11 | do | Roots | do | do | 7 | 12.30 p. m. | | 1.00 | | | 35.55 | | | | | | .237 | |
| 12 | Normal | Leaves | Pulp | Next east of factory | 15 | 8.00 a. m. | 7.00 | 10.83 | 17.83 | 2.97 | 26.11 | 15.85 | 18.82 | 3.11 | 1.89 | .84 | | |
| 13 | do | do | Juice | do | 15 | 8.00 a. m. | | 7.04 | | | 33.58 | | | 4.63 | | | | |
| 14 | do | Roots | Pulp | do | 15 | 8.00 a. m. | 15.50 | 6.29 | 21.79 | .735 | 7.62 | 2.20 | 2.94 | 1.36 | .392 | 64.20 | | |
| 15 | do | do | Juice | do | 15 | 8.00 a. m. | | 1.10 | | | | | | | | | | |
| 16 | do | Leaves | Pulp | Chandler farm | 16 | 5.00 a. m. | 3.00 | 9.55 | 12.55 | 3.98 | 19.80 | 15.08 | 19.06 | 5.31 | 4.05 | 1.24 | | |
| 17 | do | do | Juice | do | 16 | 5.00 a. m. | | 5.88 | | | 24.70 | | | 7.61 | | | | |
| 18 | do | Roots | Pulp | do | 16 | 5.00 a. m. | | 5.52 | | | 9.12 | | | 1.42 | | | | |
| 19 | do | do | Juice | do | 16 | 5.00 a. m. | | .44 | | | 24.35 | | | | | | | |
| 20 | Apparently healthy; carrying seed; siloed over winter. | Leaves | Pulp | Garden east of station. | 16 | 10.00 a. m. | 4.50 | 13.20 | 17.70 | 3.70 | 27.76 | 20.65 | 24.35 | 3.28 | 2.44 | 1.58 | | |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 21 | ....do.... | ....do.... | Juice.... | ....do.... | 16 | 10.00 a. m. | ...... | 9.08 | ...... | ...... | 37.42 | ...... | ...... | 4.27 | ...... | ...... | ...... | ...... |
| 22 | ....do.... | Roots.... | Pulp.... | ....do.... | 16 | 10.00 a. m. | 11.15 | 7.70 | 18.85 | .45 | 10.92 | 4.47 | 4.92 | 1.39 | .567 | 46.45 | ...... | ...... |
| 23 | ....do.... | ....do.... | Juice.... | ....do.... | 16 | 10.00 a. m. | ...... | 1.76 | ...... | ...... | 33.82 | ...... | ...... | ...... | ...... | ...... | ...... | ...... |
| 24 | ....do.... | Leaves.... | Pulp.... | ....do.... | 17 | 7.30 a. m... | 3.50 | 11.46 | 14.96 | 1.90 | 28.42 | 21.79 | 23.69 | 3.09 | 2.37 | 1.44 | ...... | ...... |
| 25 | ....do.... | ....do.... | Juice.... | ....do.... | 17 | 7.30 a. m... | ...... | 6.18 | ...... | ...... | 35.52 | ...... | ...... | ...... | ...... | ...... | ...... | ...... |
| 26 | ....do.... | Roots.... | Pulp.... | ....do.... | 17 | 7.30 a. m... | 7.40 | 5.89 | 13.29 | 1.77 | 11.78 | 5.22 | 6.99 | 1.08 | .48 | 35.62 | ...... | ...... |
| 27 | ....do.... | ....do.... | Juice.... | ....do.... | 17 | 7.30 a. m... | ...... | 1.00 | ...... | ...... | 33.06 | ...... | ...... | ...... | ...... | ...... | ...... | ...... |
| 28 | ....do.... | Seeds.... | Whole.. | ....do.... | 17 | 7.30 a. m... | 3.45 | 24.50 | 27.95 | .896 | 7.85 | 6.89 | 7.79 | ...... | ...... | .48 | ...... | ...... |
| 29 | ....do.... | Leaves.... | Juice.... | ....do.... | 18 | 9.00 a. m.. | 3.20 | 8.08 | 11.28 | 3.54 | 37.05 | 26.60 | 30.14 | 2.18 | 1.58 | 1.48 | .230 | 2.04 |
| 30 | ....do.... | Pedicels and midribs. | ...do.... | ....do.... | 18 | 9.00 a. m.. | 4.10 | 3.84 | 7.94 | 9.08 | 38.36 | 18.56 | 27.64 | ...... | ...... | 5.60 | .173 | 2.18 |
| 31 | ....do.... | Seeds.... | ...do.... | ....do.... | 18 | 9.00 a. m.. | 3.60 | 4.40 | 8.00 | 1.15 | 22.44 | 12.35 | 13.50 | ...... | ...... | 4.27 | .590 | 7.38 |
| 32 | ....do.... | Upper half of roots. | ...do.... | ....do.... | 18 | 9.00 a. m.. | 14.70 | .90 | 15.60 | 1.38 | 24.01 | 1.38 | 2.76 | ...... | ...... | 83.95 | .144 | .92 |
| 33 | ....do.... | Lower half of roots. | ...do.... | ....do.... | 18 | 9.00 a. m.. | 12.00 | .80 | 12.80 | 4.15 | 30.63 | 1.92 | 6.07 | ...... | ...... | 78.00 | .237 | 1.81 |
| 34 | Curly-top.... | Roots.... | Pulp.... | Large Government field. | 21 | 8.00 a. m.. | 12.40 | 6.53 | 18.93 | 1.08 | 11.59 | 3.99 | 5.07 | 1.71 | .589 | 55.70 | ...... | ...... |
| 35 | ....do.... | ....do.... | Juice.... | ....do.... | 21 | 8.00 a. m.. | 15.60 | .96 | 16.56 | 2.30 | 27.99 | 1.62 | 3.92 | ...... | ...... | 71.90 | .252 | 1.52 |
| 36 | Healthy.... | Large leaves.... | Pulp.... | Chandler farm.... | 22 | 7.00 a. m.. | 3.90 | 9.35 | 13.25 | 2.11 | 18.32 | 12.95 | 15.06 | 4.77 | 3.37 | .92 | ...... | ...... |
| 37 | ....do.... | ....do.... | Juice.... | ....do.... | 22 | 7.00 a. m.. | 4.00 | 5.50 | 9.50 | 5.75 | 22.98 | 13.30 | 19.05 | 7.19 | 4.15 | 1.17 | .184 | 1.94 |
| 38 | Plant with small leaves; otherwise normal. | Small leaves.... | ...do.... | ....do.... | 22 | 7.00 a. m.. | 3.90 | 5.80 | 9.70 | 4.02 | 29.32 | 17.50 | 21.52 | ...... | ...... | ...... | .499 | 5.15 |
| 39 | Curly-top.... | Roots.... | Pulp.... | Large Government field. | 22 | 1.00 p. m.. | 13.90 | 7.02 | 20.92 | 6.94 | 10.38 | 3.48 | 10.42 | 1.85 | .021 | 54.10 | ...... | ...... |
| 40 | ....do.... | ....do.... | Juice.... | ....do.... | 22 | 1.00 p. m.. | 16.45 | 1.14 | 17.59 | 1.54 | 21.55 | 1.39 | 2.93 | ...... | ...... | 80.25 | .184 | 1.05 |
| 41 | ....do.... | Leaves.... | Pulp.... | ....do.... | 22 | 1.00 p. m.. | 4.20 | 10.07 | 14.27 | 4.10 | 19.11 | 13.50 | 17.60 | 4.81 | 3.40 | .52 | ...... | ...... |
| 42 | ....do.... | ....do.... | Juice.... | ....do.... | 22 | 1.00 p. m.. | 3.70 | 6.68 | 10.38 | 3.56 | 19.88 | 12.82 | 16.38 | 6.57 | 4.24 | .99 | .388 | 3.74 |
| 43 | Large healthy plant... | Leaves 12-18 inches long. | ...do.... | Chandler farm.... | 23 | 1.00 p. m.. | 4.50 | 6.88 | 11.38 | 4.22 | 28.06 | 16.98 | 21.20 | 6.18 | 3.74 | .62 | ...... | ...... |
| 44 | ....do.... | Upper half of root. | ...do.... | ....do.... | 23 | 1.00 p. m.. | 15.90 | .66 | 16.56 | 1.06 | 20.21 | .806 | 1.87 | ...... | ...... | 57.00 | ...... | ...... |
| 45 | ....do.... | Lower half of root. | ...do.... | ....do.... | 23 | 1.00 p. m.. | 16.85 | .72 | 17.57 | 1.82 | 23.52 | .96 | 2.78 | ...... | ...... | 75.00 | ...... | ...... |
| 46 | Small plants; retarded by excessive watering. | Leaves.... | ...do.... | ....do.... | 23 | 1.00 p. m.. | 4.58 | 7.23 | 11.81 | 1.72 | 25.20 | 15.42 | 17.14 | 7.02 | 4.30 | 1.61 | ...... | ...... |
| 47 | ....do.... | Upper third of roots of No. 46. | ...do.... | ....do.... | 23 | 1.00 p. m.. | 14.25 | .92 | 15.17 | 1.75 | 26.56 | 1.61 | 3.36 | ...... | ...... | 82.00 | ...... | ...... |
| 48 | ....do.... | Lower third of roots of No. 46. | ...do.... | ....do.... | 23 | 1.00 p. m.. | 15.15 | .90 | 16.05 | 2.24 | 29.11 | 1.63 | 3.87 | ...... | ...... | 77.00 | ...... | ...... |
| 49 | Curly-top.... | Leaves.... | ...do.... | Government field.... | 25 | 2.00 p. m.. | 4.95 | 6.42 | 11.37 | 8.80 | 26.82 | 15.22 | 24.02 | 5.25 | 2.96 | .49 | .288 | 2.54 |
| 50 | Healthy seed-bearing plants. | Seeds.... | ...do.... | Garden east of station. | 26 | 2.00 p. m.. | 4.90 | 6.40 | 11.30 | 6.55 | 23.53 | 13.35 | 19.90 | 4.69 | 2.65 | .97 | .583 | 5.16 |
| 51 | Large healthy plants.. | Large leaves.... | ...do.... | Field east of factory... | 30 | 6.10 a. m.. | 3.10 | 6.94 | 10.04 | 1.60 | 33.78 | 23.35 | 24.95 | 5.05 | 3.48 | 1.48 | .260 | 2.59 |
| 52 | ....do.... | ....do.... | ...do.... | ....do.... | 30 | 9.30 a. m... | 3.65 | 8.06 | 11.71 | 2.38 | 34.32 | 23.62 | 26.00 | 4.61 | 3.17 | 2.03 | .260 | 2.22 |
| 53 | ....do.... | ....do.... | ...do.... | ....do.... | 30 | 2.45 p. m... | 5.38 | 10.15 | 15.53 | 2.26 | 31.05 | 20.30 | 22.56 | 5.89 | 3.84 | 3.06 | .310 | 2.00 |

## CHEMICAL ANALYSIS OF SAMPLES COLLECTED IN THE FIELD.

As stated above, samples of diseased and of healthy sugar beets were collected and brought to Washington for analysis. When the juice was to be preserved 100 grams of it were mixed with 150 cubic centimeters of 95 per cent alcohol. In some cases it seemed desirable to preserve some of the pulp from which the juice had been expressed. Such pulp samples were weighed and preserved in wide-mouthed bottles with two volumes of alcohol. The moisture, ash, total nitrogen, and cane sugar were determined in most of the samples. Unfortunately, a few of them did not afford enough material to complete the analysis. The determinations of cane sugar, ash in the alcohol-insoluble material, and nitrogen were made by the Bureau of Chemistry, and the results are shown in Table XIX (p. 24).

The figures reveal no parallelism between the concentration of the other constituents estimated and the condition of the plant, apart from the oxidase content of the leaves. The increased concentration of oxidases in the plants of inhibited growth becomes apparent from the two columns R and S under "Oxidases." Column R gives the concentration of the oxidases in the juice of the leaves, expressed in terms of units (pyrogallol). The figures in column S are obtained by multiplying the units of column R by 100 and dividing by the percentage of solids in the particular sample (shown in column J). The data for oxidase contents were thus reduced to an "all-solids" basis in order to ascertain whether the variations in oxidase concentration in some of the cases were due to a variation in the moisture content of the samples. In samples 51, 52, and 53 these computations changed the entire aspect of an experiment. The oxidase content of the juice of leaves which increased from 0.26 to 0.31 units (pyrogallol) during the period from 6 a. m. to 2.45 p. m., in reality diminished to four-fifths of its value at 6 a. m. when expressed as a fractional part of all the solids present. The rise of oxidase content shown in series 13 and 14 would probably also vanish if results were reduced to the "all-solids" basis, but unfortunately no samples were collected in connection with these two series of experiments.

## DISCUSSION OF RESULTS.

The experiments described in this paper corroborate the results obtained with curly-top sugar beets grown in the greenhouse (Bunzel, 1912). The leaves of the curly-top plants had an oxidase content two to three times as great as the healthy and normally developed ones. No marked differences, however, could be detected between the roots of the two kinds of plants. An abnormally high oxidase content of leaves was also shown in plants whose growth

had been retarded. When the plants were stunted either by excessive watering, by drought, or through unknown agencies, the oxidase content of the leaves was much higher than in normal and healthy plants. The increase in oxidase concentration in the leaves is not a mere function of their size, inasmuch as very young normal leaves do not exhibit this characteristic. If the condition of the plant is such that only one of its functions (as the development of seed in biennially grown beets) is inhibited, the oxidase content is also high. The most general conclusion to be drawn from these observations is that an abnormal retardation of growth in sugar-beet plants is accompanied by an increase in the concentration of oxidases in the leaves or a change in the juice of the latter by which the pyrogallol oxidizing oxidase becomes more active.

Such an increase in the power of the juice to bring about or hasten oxidation under pathological conditions has been observed before. Woods found it in connection with a disease of tobacco, Sorauer in connection with the leaf-curl of potatoes. Further investigation must show whether the oxidases which have been studied by the authors cited and by the writer are identical with or directly related to those which Palladin and his school find so important in the respiration of plants. If they are, it is probable that an increase in their concentration leads to increased combustion in the cells. One would then be tempted to look at such plants as in a state of "fever."

The moisture, ash, sugar, and nitrogen content of some of the samples examined show no parallelism between gross chemical composition on the one hand and the extent of the disease and the oxidase content on the other. Moisture determinations are essential where experiments on the same plant at different times of the day are to be compared. The oxidase content of the juice increases in the course of the day, but slightly diminishes when calculated on the basis of the total solids present. In general, it may be said that the results obtained on normal material are more irregular and somewhat higher than the results previously obtained on the normal greenhouse material.

The distribution of the oxidase accomplishing the rapid oxidation of pyrogallol through the sugar-beet plant was studied. All of the parts examined were active. The juice of the seeds had the greatest oxidizing power, the juice of the leaves and roots being next in activity. The lower portion of the root was more active than the upper portion, being sometimes twice as active. The juice of the pedicels and midribs was almost as active as the leaf juice. The stem furnished a juice feebler in activity than any other part of the plant. In the green parts of the plant there seemed to be a general parallelism between the oxidase activity and the depth of the green color.

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